

On the Biology of Sexual Reproduction of Hydromedusae, Genus *Phialidium* Leuckhart¹

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THE GENUS *Phialidium* is nearly ubiquitous in the coastal waters of the temperate zone. The medusae occur at Friday Harbor certainly from April through September and disappear rather suddenly in October, under circumstances which need investigation. Drifting with tides and currents, they occur in swarms which greatly vary in density. During the present investigation no swarms were seen in which individuals, on the average, were closer to each other than a few inches; usually they were many inches to several feet apart. Under these circumstances it would seem that fertilization becomes somewhat of a problem. In a population which, as a whole, is moving passively, and in which individuals appear to have no affinity to each other, ovulation and spermiation must be closely and appropriately timed and the properties of eggs and sperms evolved to insure a high rate of fertility, the results of which are seen in the wide distribution and in the tremendous numbers of colonies of the sessile stages of the species, the hydroid polyps. The present paper attempts to clarify some of the factors which insure the high reproductive capacity of hydromedusae under what, on first sight, appear to be difficult circumstances.

MATERIAL AND METHODS

Among the hydromedusae found at Friday Harbor there are apparently two species of *Phialidium*. One of these, used infrequently in the present investigation, is almost certainly *P. hemisphaericum* (L.). It conforms in all essentials to the description given by Russell (1953: 287).

¹ Aided by a grant from U.S. Public Health Service, RG-4714. Manuscript received May 20, 1960.

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The second form of *Phialidium*³ was found much more frequently at Friday Harbor in August 1959 and was used prevalently in the present work. It has usually been called *P. gregarium* (L. Agassiz). It could be clearly distinguished from *P. hemisphaericum* by its size, maximum 20–22 mm., the number of tentacles, up to 60, the number of statocysts, 90–120, by a thicker umbrella, a more viscous jelly and more folded lips (Fig. 1). Recently, the species has been redefined by Kramp (1962) who has pointed out the reasons for designating it as *P. gregarium*, although its original description by Agassiz must be considered inadequate.

The animals used in this investigation were almost all caught during the month of August 1959 from the dock at the Friday Harbor Laboratories at various times of the day between 0800 and 2200 hours. In the evening hours a nightlight was used in the water. The animals were taken out in glass bowls, never by hand or by net, and throughout the investigations were transferred in water by means of plastic spoons or small bowls. As a consequence of the method of catching, only animals floating near the surface (not deeper than 1 ft.) were used. In the laboratory the animals were kept in dishes of 50–1200 cc. capacity. The dishes were maintained on a water table in running sea water which kept the temperature between 11° and 13° C. The animals themselves were not kept in running water but the water was usually renewed twice a day or more often. By using only new utensils which had been soaked for at least a few days in running sea water before use, chemical contamination was held at a minimum (the pipe lines at Friday Harbor are nonmetal).

³ The term "*Phialidium* species" used throughout this paper should be construed to mean *Phialidium gregarium*.

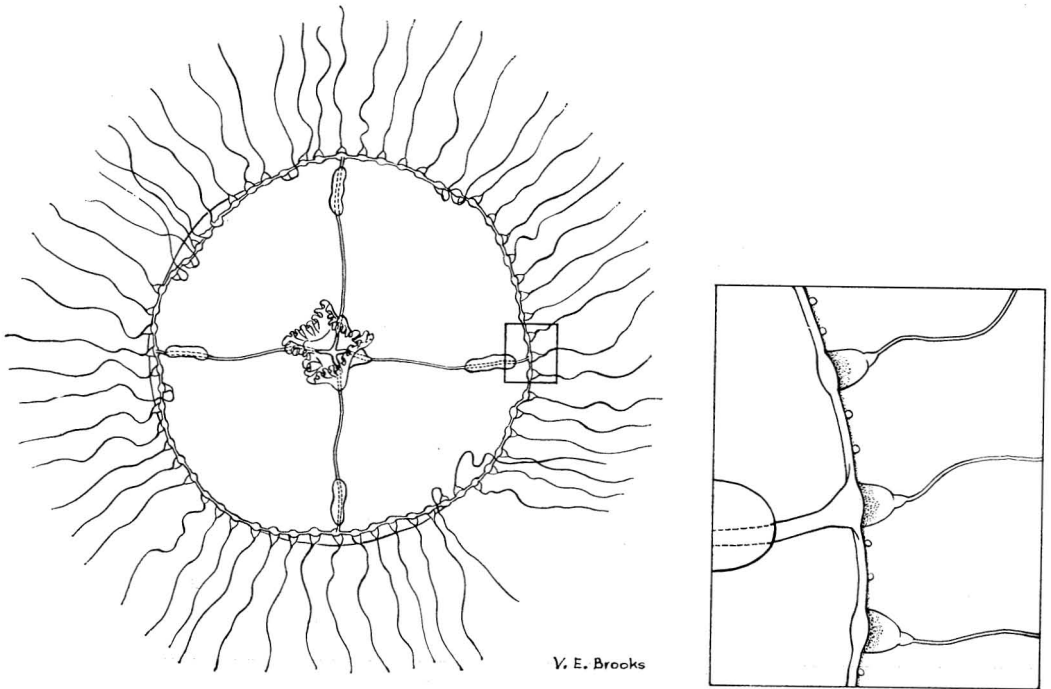


FIG. 1. *Phialidium* species. Semidiagrammatic drawing of subumbrellar view. Magnification approx. $3\times$.

Light conditions in the laboratory during the daytime were similar to those found in the upper layers of the sea outside. At night, for many hours after sunset, it often was much lighter in the laboratory than under natural conditions.

Moving the animals, and especially transferring them in spoons, often caused violent contractions of their bells and they frequently remained contracted for many seconds, but these rare shocks are not dissimilar to those which an animal may receive in nature by being thrown about in waves and tidal currents. A more unnatural condition was introduced by the lack of irritation during the long periods in which more or less isolated animals were kept in standing water. If a medusa lives under such conditions it usually becomes very quiet, pulsates only infrequently and may even turn upside down and lie almost motionless for a half-

minute or longer at the bottom of the dish. When moved or touched it will immediately begin to pulsate again. It was not investigated whether the periods of enforced quiet had any influence on the reproductive behaviour.

No attempt was made to feed the animals in the laboratory. All animals were freshly caught a few hours before they were used and no animal was used in experiments for longer than 48 hr. Although microplankton was available in the dishes the animals rarely had any visible stomach contents at the end of the experiments, but neither did they show any effects of lack of food. Nearly all animals which were kept for days or weeks survived but after some days began to regress. The atrophy became visible first in the gonads but later in all structures of the medusa. After 7–10 days the animals showed a marked decrease in size and remained motionless unless stimulated.

Two simple techniques were used in experiments: (1) *Artificial periods of darkness* were introduced by a simple box which was put over the dishes. This box had an inlet and outlet under water through which circulation was maintained and the animals were thus kept at the same temperature as those outside the box. (2) *Sperm counts* were made of water in which males had been present. Hemocytometers (B & L) were used for counts. Preliminary studies to determine the reliability of the technique revealed that results were reasonably consistent, provided that the operator was able to identify the spermatozoa at a magnification of approximately $400\times$. They can be identified when either the flagellum is visible or when the size and shape of the refractile head, often together with a characteristic motion, can be recognized. In the final counts spermatozoa were counted only when these two sets of criteria were verified singly or in combination. Sperm were counted in the whole area of the chamber, 1 mm.^2 Usually no dilution of the water containing sperm was necessary. The water was stirred thoroughly, and sucked into a leucocyte pipette, and shaken briefly; the first two drops were discarded and the chamber was filled. Long shaking proved to be unnecessary because the spermatozoa of *Phialidium* show no tendency to congregate or coagulate. In fact, the relative ease and reliability of the method appeared to rest in great part on these characteristics.

Many observations were made with the dissecting microscope at magnifications between $25\times$ and $50\times$. In addition, the gonads were often studied in anesthetized animals (chloretone-magnesium chloride was added until the animals remained quiet) with the phase microscope, usually under a magnification of $400\times$ but occasionally up to $1,000\times$. Histological preparations were made of the gonads. A battery of fixatives, including acrolein at room temperature and chilled 1 per cent potassium permanganate, was tried. However, the more conventional methods—such as Bouin's solution in sea water or 10 per cent formalin for 1–2 hr. with after-fixation for 1–2 hr. in Helly's or Maximow's—gave better results. The preparations ($3\text{--}6\text{ }\mu$ thick) were stained with Heidenhain's Iron-Hematoxylin, Heidenhain's Azan, or with the PAS reaction.

RESULTS

Observations and Experiments on Ovulation

Every female *Phialidium* with intact gonads brought into the laboratory during August 1959 released eggs at least once within the next 24 hr. In several cases ovulation was observed directly, but usually the approximate time of ovulation was calculated from the age of the developmental stages of the eggs observed at varying times after ovulation. The data used were derived from many observations of developing eggs and are summarized in Table 1. After ovulation the outline of the ovary is smooth and the eggs appear compressed into the confines of the "linear" gonad. Several hours before ovulation the eggs to be ovulated can be recognized because they begin to bulge at the surface of the ovary. At first the ovary presents a slightly wavy surface but shortly before ovulation the eggs have completely protruded and appear to hang like grapes from a stalk. Ovulation usually takes place within 10–15 min. The majority of the eggs are released in less than 5 min. In a fully grown and well-fed specimen a total of 50–70 eggs may be released, in smaller animals only 10 to 20, but occasional ovulations of only 1 egg or of very few ova were also observed.

The eggs are very gently released from the ovary without any motion which would indicate that they are propelled. However, as soon as they become free in the subumbrellar cavity, they are rapidly dispersed by means of the pulsations of the animal. Usually, the females are very actively pulsating during the period of ovulation as though they were somewhat irri-

TABLE 1
TIME TABLE FOR CLEAVAGE STAGES OF
Phialidium SPECIES AT $11^{\circ}\text{--}13^{\circ}\text{ C.}$

FROM OVULATION AND IMMEDIATE FERTILIZATION	
To Beginning of:	Average Times
1st cleavage	50 min.
2-cell stage	70–90 min.
2nd cleavage	95 min.
4-cell stage	110–120 min.
8-cell stage	150 min.
approx. 16-cell stage	180 min.
approx. 32-cell stage	210 min.

tated. Two females were observed in contraction during the height of ovulation, in the same way in which animals contract which have been violently touched. These females were lying upside down for about a minute, during which many eggs fell slowly from the ovary into the subumbrellar cavity. When the animals began to pulsate normally again they righted themselves and immediately scattered the eggs widely into the water.

In the majority of cases ovulation took place twice within 24 hr., once at night after sunset and once in the morning before sunrise. Nineteen dishes containing 2–6 females each were studied at various times through 24 hr., and the observed ovulations and eggs indicated that in every case at least 1 female, often all, had two ovulation periods. In 45 different cases, individual females were observed through at least 24, sometimes through 48 hr. Twenty-one had an evening and a morning ovulation and all 5 animals which were observed for another day showed another evening and morning ovulation. Twenty animals had only an evening ovulation. Of these, 5 were observed for 24 hr. longer and 3 continued in the same pattern with evening but no morning ovulation. The other 2 had an evening and a morning ovulation on the second day. Four females had only a morning ovulation on the first day and 1 of these, observed for another day, then had only an evening ovulation.

In 32 individual cases the ovulation time was determined (Fig. 2). It was found that ovulations occurred from 55 min. before to 4 hr. after sunset, with the majority of cases between 1 and 3 hr. after, and from $1\frac{3}{4}$ hours before to $2\frac{3}{4}$ hours after sunrise, with most ovulations occurring about 1 hr. before. Many other cases not recorded in detail appeared to fall within these limits—with a few notable exceptions. Two animals shed a few eggs around noon; in each case there had been several transfers immediately before ovulation. There were other occasional observations of single eggs being released at almost any time of the day.

In those cases in which both evening and morning ovulations were observed, the evening ovulation nearly always produced the greater number of eggs. Furthermore, eggs were almost

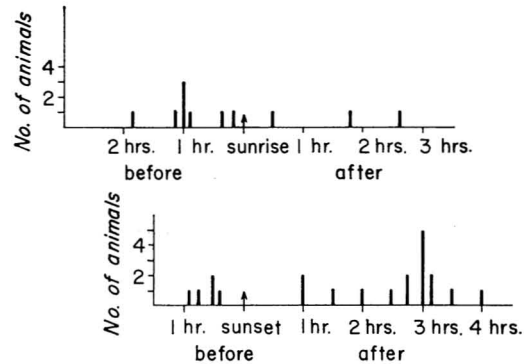


FIG. 2. Ovulation times of *Phialidium* species.

100 per cent fertilized in the evening but only 90 per cent or less in the morning.

Only a few individuals of *P. hemisphaericum* were thoroughly investigated. It appeared that this species also usually ovulated after sunset and in the morning, but in 6 cases in which the morning ovulation was timed precisely it occurred 2–3 hr. later than in the animals of *P. species* observed on the same mornings.

Several series of experiments were performed to ascertain in a general way the effect of light on the times of ovulation. Of these six will be recounted as examples.

Experiments with Morning Ovulation

NO. 1: Nine pairs of *P. species* were kept separate, darkened around the time of sunset (1915), until the dark box was removed at 0510, about 20 min. after sunrise. Five females had ovulated late at night, as did all of the 4 controls. Of these 5 only 3 ovulated again on the following morning, at 0555 and two at 0630. The 4 females which had not ovulated at night released eggs at 0515 and 0530. One female ovulated at 0400, the same time as all 4 controls. The dish containing this animal was found to receive some light through a drainage hole.

NO. 2: Five pairs of *P. species* were kept separate, darkened around the time of sunset (1920), until the dark box was removed at 0600, $1\frac{1}{4}$ hr. after sunrise. All females laid eggs late at night, as did the 4 controls. All females had morning ovulations between 0700 and 0830; 3 of the controls ovulated between 0400 and 0530, 1 did not ovulate in the morning.

NO. 3: Three pairs of *P. species* were kept separately and darkened around the time of sunset (1910). The dark box was removed at 0735, about 3 hr. after sunrise. All 3 females laid eggs at 0900, 1 at 1000, and 1 had apparently released eggs over a protracted period from 0500 to 0630 while still in the dark. The 2 controls ovulated around 0400.

These experiments indicate an effect of light on morning ovulation. In 13 out of 17 cases, ovulation occurred much later than in the controls. Ovulation occurred 5 min. to 3 hr. after exposure to light, with 7 cases ovulating after 1½ to 2½ hr. Intervals were very short only in the experiment (No. 1) in which the difference between artificial exposure to light and sunrise was not great.

Experiments with Evening Ovulation

NO. 4: Six pairs of *P. species*, 2 pairs to a dish, were darkened from noon until 1645. One female ovulated at 1445, 2 at 1600, the other 3 between 2000 and 2200. Three controls ovulated between 2100 and 2200.

NO. 5: Four pairs of *P. species*, kept separate, were darkened at 1300, 4 were darkened at 1500. The experimental animals were kept in the dark until the dawn on the next morning, but were briefly subjected to light on four occasions during the evening. Four controls laid eggs at night, 2 at 2000, and 2 at 2150; only 1 control ovulated the next morning, at 0700. One control which had laid a few eggs at noon immediately after having been caught from the sea, shed 2 eggs at 1500 and 3 eggs around 1700. All of these developed normally. The experimental animals which were darkened at 1300 behaved essentially like the controls; 2 laid eggs around 2200, 2 between 2300 and midnight. Three of the animals put in the dark at 1500 ovulated around 1830 and again in the morning between 0330 and 0500, 1 ovulated at 2230 and had no morning ovulation. Fertilization and development of the eggs appeared normal in all cases.

NO. 6: Series A consisted of 4 pairs, kept separate, which were put into the darkbox at 1600. Series B, also 4 pairs, were treated in the same way as Series A; these were animals used in experiment No. 5, which had been darkened

at 1500 on the previous day. Four controls were kept, of which 2 had been used as controls on the day before. The last ovulated between 2230 and 2300 but not on the following morning; of the other controls, 1 ovulated at 2300 and 0830 the other at 1800 and around 0500.

All animals of Series A laid eggs between 1930 and 2000 and 1 female ovulated in the morning around 0830. Those of Series B ovulated at 2030, 2100, and 2115, and 1 had no evening ovulation but shed eggs at 0430.

These experiments, and others not reported in detail, indicate an effect of darkening on evening ovulation depending on the time of day at which the animals are put in the dark. Of 10 animals darkened at noon or 1300, 2 ovulated after 2½ to 4 hr. Of 4 animals darkened at 1500, 3 ovulated 3½ hr. later. All 4 animals darkened at 1600 ovulated 3½–4 hr. later. Animals which appeared to respond to darkening at 1500 on one day seemed to respond more slowly to darkening at 1600 on the next day: 3 out of 4 shed eggs 4½ to 5¼ hr. after darkening. There was no indication that brief exposure to light, which in some experiments took place after the period of dark, had any influence on ovulation.

Observations and Experiments on Sperm Production

Spermiation (sperm release) involving small numbers of spermatozoa was repeatedly observed. When males were closely scrutinized around the usual ovulation times of the females, the surface of the testes was sometimes seen to be uneven in places. Under the phase microscope at magnifications around 400 × it was observed that the surface cells in such areas appeared thickened and bulging. Spermatozoa in small numbers appeared to move slowly in a smooth and almost continuous stream through breaks in the surface. Within the testis the flagella of the sperm cells showed a low degree of motility but the flagellar motion increased as the spermatozoa became free of the gonad. Immediately after the release the flagellar beat of the epithelial cells of the gonadal surface swept the spermatozoa away. Whenever males were closely observed, some living spermatozoa were found moving about in the vicinity of the gonad even though actual spermiation was not always seen.

TABLE 2
AVERAGE TOTAL SPERM COUNTS PER MALE FOR
VARIOUS INTERVALS OF TIME, BEGINNING
AT 1600 OR 1700

EXPERI- MENT	NO. OF MALES	INTERVAL (hours)	TOTAL COUNT PER MALE IN 10^6
1	8	2½	1.2
	8	19	2.5
2	8	17	2.4
	8	26	1.2*
3	4	14	3.6
	4	16	3.6
4	4	18	2.9
5	4	1	0.4
	4	19	2.9
6	2	1	0.4
7	1	1	0.5
8†	1	2½	1.1
	1	16	2.2

* Many dead spermatozoa seen.

† Data from animal A of Fig. 3, to show comparability.

Some years ago a major sperm release was once accidentally observed in which dense clouds of spermatozoa issued from the gonads of an animal about the time of sunrise. In the present investigation many attempts were made to observe a major sperm release in the late evening and early morning hours but all were unsuccessful. The number of spermatozoa observed to leave through small breaks in the gonadal surface never exceeded one or two hundred.

It was observed that sperm cells dispersed rapidly. Their motility was extremely vigorous in fresh preparations. When the spermatozoa were confronted with small obstacles the speed and excursion of flagellar activity was remarkable. Even hours after spermiation spermatozoa were often seen to move energetically in the counting chamber.

Results of sperm counts are demonstrated in Table 2 and Figure 3. In studying the data it should be kept in mind that every 10,000 spermatozoa in the total counts correspond to 1 single spermatozoon actually counted. The accuracy of the individual counts is, therefore, not high, particularly in the lower range. However, the data in Table 2 are fairly consistent throughout and indicate an approximate rate of production of at least 3,000,000 sperm per day on the average.

The data presented in Figure 3 appear to confirm what direct observations indicated, that sperm release may occur in small amounts at almost any time of day or night but that there are major periods of release in the late evening and early morning hours. There appear to be great individual variations in this phenomenon. In all probability major periods of spermiation are brief.

Sperm Concentrations and Fertile Age of Eggs and Sperms

A number of experiments were performed which indicated that spermatozoa survived and were fertile in considerable dilution. Females were transferred through several changes of filtered sea water and kept in filtered sea water in order to exclude the accidental presence of spermatozoa. Sperm was added in various dilutions and at various times before and after ovulation. For instance, approximately 4 cc. out of 250 cc. of sea water in which one male had been swimming from 1720 on were added at 2200 to a bowl containing freshly laid eggs in 250 cc. of water. On the next morning at 0600 about 50 eggs were found in normal development. Four similar experiments were performed on various days with essentially the same re-

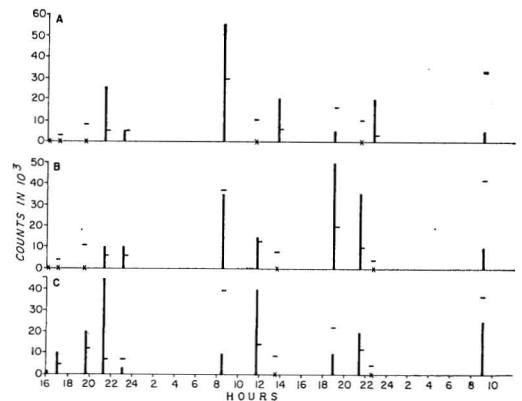


FIG. 3. Sperm counts of 3 different males of *Phialidium* species. Each column represents the amount of spermatozoa shed since the last count (after each count there was a thorough change of water).

x = count 0.

- = theoretical count if sperm had been shed continuously and in equal amounts over the whole period of observation for the respective male.

sults. In the absence of sperm counts it may be assumed that the water in which the male had been swimming for 4 hr. and 40 min. contained at least 1.5 million spermatozoa or 6,000/cc. This means that at least 24,000 sperm were added to the 250 cc. of water containing the eggs. Fertilization presumably took place at an average sperm concentration of about 100/cc. This concentration appeared ample as failures were not recorded in these 5 experiments. Concentrations of approximately 2 spermatozoa/cc. were always unsuccessful, while concentrations between 5 and 80/cc. were successful in varying degrees. For instance, in one experiment a drop (about 0.05 cc.) out of 250 cc. of water in which 8 males had been placed for 12 hr. was added to 88 eggs in 130 cc. of water. Eight of the eggs were fertilized and developed normally. If it is assumed that 8 males produced about 16 million spermatozoa in 12 hr. (Table 2) then approximately 30,000 sperm were added to the eggs and acted in a concentration of 32/cc.

At all concentrations of less than 100 spermatozoa/cc. fertilization occurred over a period of time which extended over 2½ to 3 hr. (as estimated by the appearance of the first furrow). In these cases the eggs which began development last often developed abnormally. It was possible, however, in some cases to fertilize eggs with higher concentrations of sperm until 4½ hr. after ovulation; these eggs showed a high proportion of normal embryos.

When sperm which was 10 hr. old was used in high concentration to fertilize fresh eggs, less than 50 per cent of the eggs developed and these began development slowly, ½ hr. after ovulation. The age of the sperm below 10 hr. did not appear to make an appreciable difference in the rate of fertilization. In one case a few eggs developed normally after having been fertilized with spermatozoa nearly 12 hr. old.

Some Notes on the Histology of the Gonads

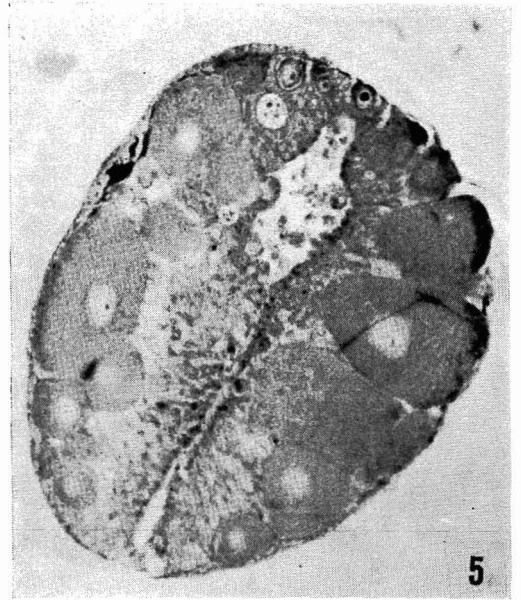
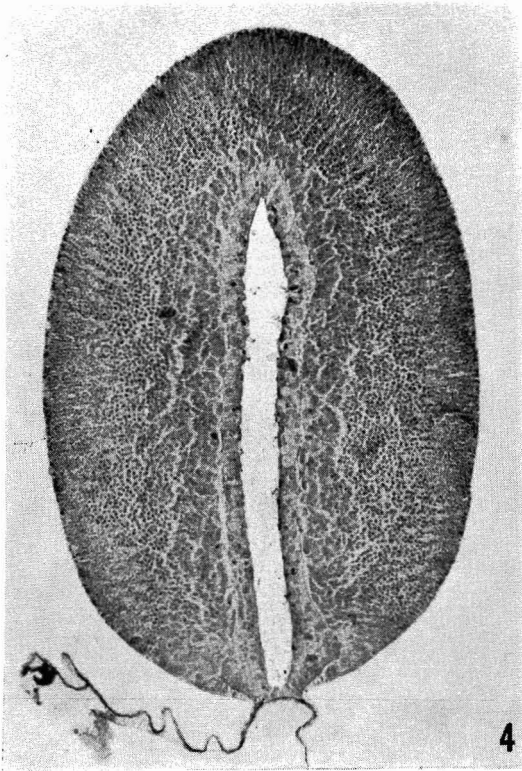
There are very few observations on the internal structure of the gonads of hydromedusae in the literature. The most extensive observations were made by O. and R. Hertwig (1878: 27), whose figures depict many of the features found in the present investigation. Brooks

(1888) and Mayer (1910: 271) have described the process by which, in *P. McCradyi*, hydroid blastostyles develop in the region of the gonads, and they have given casual descriptions of the normal structures. As a consequence of the small amount of research done on the subject, the prevailing view today is that "the gonads in hydromedusae can scarcely be designated organs, being merely accumulations of sex cells in definite sites" (Hyman, 1940: 431). The following brief account of the histology of the gonads of *Phialidium* is a preliminary survey and is appended here only because a basic knowledge of the structure of the ovary and particularly of the testis is helpful in discussing the biology of reproduction. In a future publication a detailed and more quantitative account of gametogenesis in *Phialidium* will be given.

The gonads in *Phialidium* (Figs. 4, 5) are situated at the subumbrellar side around the radial canals. The canals not only widen inside the gonad but their gastrodermal lining takes on a special character. It is much taller and more complex in structure throughout the extent of the gonad. This specialized epithelium is most developed in the most active gonads and regresses in correlation with regression of the gametogenic tissues.

In the male, the gastroderm is delimited toward the gonad by a conspicuous basement membrane which is PAS positive and stains strongly with aniline blue. The membrane appears somewhat scalloped, with fine processes pointing at regular intervals toward the surface of the testis (Fig. 4). In the female the gastrodermal cells often extend among the gametes, surrounding them in places and even appearing to attach themselves to their surface.

In both sexes the gonads are covered with a pigmented epithelium composed of fairly large cells. The degree of pigmentation varies; it is greater in *P. hemisphaericum* than in *P. species*, and the pigment granules appear larger and more frequent in males than in females. In the ovary this epithelium is clearly demarcated in most areas by a PAS positive basement membrane. In the testis the morphology of the surface cells is complex and has not been elucidated in full detail. It was observed, however, that the nuclei often lie considerably below the surface



FIGS. 4, 5. Cross-section through testis of *Phialidium* species, with a small piece of subumbrellar epithelium attached; PAS stain, $6\ \mu$ thick; magnification $115\times$. 5, Cross-section through ovary of *Phialidium* species; PAS stain, $6\ \mu$ thick; magnification $115\times$.

and that columns of cytoplasm appear at intervals to extend from the surface and in some cases, perhaps in all, reach the basement membrane of the gastroderm with more or less tenuous processes. Along these processes of the surface epithelium the spermatids appear ordered. The flagella generally point in bundles toward the basement membrane. In the testis the gametes are arranged in definite layers within which the germ cells are often in the same stage of development over large areas. A generation of large spermatogonia is situated near the basement membrane; next in the direction toward the surface is another layer of smaller spermatogonia, then follows a layer of spermatocytes and one of spermatids. The thickness of the last layer varies greatly. In the ovary (Fig. 5) the gametes are not aligned in definite order. The largest oocytes usually bulge on the

surface and stretch the surface epithelium to a very thin layer.

DISCUSSION

Apparently it has not been reported before that *Phialidium* (or any other species of Hydro-medusae) may regularly ovulate twice within 24 hr. Metschnikoff (1886 :25) stated that "*Clytia flavidula*" spawned at Naples between 8 and 9 A.M. in March and April while "*Clytia viridicans*" spawned at 8 P.M. *Clytia flavidula* is now generally regarded as identical with *P. hemisphaericum* (Mayer, 1910 :267; Russel, 1953 :293), and *Clytia viridicans* is considered to be identical with *P. buskianum*, which is possibly only a variety of *P. hemisphaericum* (Mayer, 1910 :267, 270). In any case, the two species of Metschnikoff are very closely related

to each other and to the species described in the present paper. It is difficult to understand—even if it is conceivable—that Metschnikoff did not recognize two daily periods of spawning if they occurred. The possibility must be considered that the Mediterranean forms behave differently from those observed at Friday Harbor.

Ovulation appears to be regulated within certain limits by a change in light. Metschnikoff reported Merejkowsky as the first to suggest that ovulation in *Obelia* might be stimulated by light, but Metschnikoff rejected the suggestion because of the occurrence of evening ovulation in many other species. The experiments reported in the present paper indicate that eggs will be released not only at exposure to light but whenever there is a drastic change in the lighting intensity after lighting conditions have been more or less constant for several hours. Artificial darkness, if applied after noon, will produce ovulation in the course of several hours. The later the animals are subjected to darkness the more certain ovulation will take place as a result of it. In the morning an artificial prolongation of darkness of the night will delay ovulation in most cases. There are indications that ovulation cannot be delayed indefinitely by these means, but no experiments to elucidate this point were performed.

There is as yet no direct evidence that sperm release is affected by changes in illumination. There is some evidence that major sperm releases take place at approximately the same times as ovulations. It appears, therefore, reasonable to assume that spermiation is also sensitive to photostimuli. Although the periods of spermiation have been less precisely determined, and may in fact vary more than the ovulation periods, the sequence of generations as seen in sections of the testis (Fig. 4) is remarkably orderly. In the ovary, also, several generations can be distinguished even on superficial examination, although they are not ordered in strata.

At present it is not apparent how the regular periodic sequence of generations is accelerated or delayed by light changes. Observations on animals under normal as well as experimental conditions suggest that in many cases there is a latent period between light stimulus and ovulation which may be as long as 3 hr. or more.

This interval may be long enough to permit changes in growth rate to become apparent. There are some cases, however, which show a fast response within 20 min. to 1 hr., and in these cases it must be assumed that the eggs were nearly mature and ready to be ovulated when the stimulus acted. Would they have ovulated at the same time without the stimulus?

A mechanism by which light might act on the actual release of gametes may be found in the epithelial cells covering the gonads. These contain appreciable amounts of pigment and have been observed to be contractile. It is, therefore, plausible to think of the release as initiated by metabolic changes in the surface cells which in turn bring about contraction. In the testis, contraction of the surface cells may act in two ways: the surface may become discontinuous and the contraction of the cellular processes (or some surface change along them) may release the spermatids. Live observations are in good agreement with this concept. In the ovary the surface cells have not yet been investigated in detail, but they appear to have no processes in association with the oocytes. It is certain, however, that eggs are ovulated through breaks in the epithelium.

It appears probable that light affects the rate of growth of the gametes as well as their release. Living ovaries were observed often after experimental light stimulus and were compared with controls. In general, the eggs grew faster with the stimulus than without. While the surface epithelium of the ovary has no conspicuous connections with the oocytes, the gastroderm underlying the gonad is a highly differentiated, specialized tissue containing a yellow or orange pigment which is dissolved by fat solvents. The possibility should be entertained that the nutrition of the oocytes may be changed through metabolic changes in the entoderm cells initiated by a reaction of the pigment.

The quantitative data on sperm production, crude as they are, enable us to arrive at a rough estimate of the relationship of density to fertility in swarms of *Phialidium*. If we assume that eggs are fertilized with 100 per cent probability at a concentration of 100 sperm/cc, that practically none are fertilized at 2 sperm/cc, and that a male releases about 1 million sperma-

tozoa at a major spermiation; if we further stipulate a random 3-dimensional distribution of a population composed equally of males and females, then a swarm with average distance between individuals of 18 cm. will fertilize all its eggs, while one with an average distance of 69 cm. will have practically no offspring. The average distances in swarms observed at Friday Harbor were rarely less than 25 cm. and often much greater. Mayer (1910: 270) reported that *P. languidum* crowded the harbor of Eastport, Maine, during July and September "to such an extent that their bells nearly touch as they swim at the surface of the water." In such concentrations there must be an excess of spermatozoa present if the sexes are represented equally (there is evidence that this may not be the case: at Friday Harbor occasional swarms had very unequal sex proportions). On the other hand, the prevalent density of swarms at Friday Harbor in August 1959 was such that sexual reproduction was probably very low despite the fact that huge numbers of animals were present in the waters. Wherever a meeting of gametes was successfully accomplished it was due to the combined action of the following factors favoring fertilization: survival and action of spermatozoa in great dilution, fertilizability of the ova for many hours after ovulation, and the presence of a "trigger"—light—which regulates ovulation and spermiation alike and thus brings them about at approximately the same times of day.

SUMMARY

Observations on living and histological material, simple experiments with photostimulus, and sperm counts provided the following data concerning the biology of reproduction in *Phialidium* species. and *P. hemisphaericum*:

1. Females have two ovulation periods per day, in the majority of cases: one at night some hours after sunset, one in the morning before sunrise.
2. Males release some sperm almost continually, but major periods of spermiation occur at about the times of ovulation.
3. The total number of eggs released by mature females in 24 hr. is 50–100; the total number of sperms per male is in the order of 3 million in 24 hr.
4. Spermatozoa act in great dilution and retain their vitality in dispersion. At a concentration of 100 spermatozoa per cc., 100 per cent normal fertilization was obtained.
5. Spermatozoa remain fertile for 11–12 hr. under laboratory conditions, eggs for at least 3–4 hr.
6. Artificial darkness before sunset will cause ovulation earlier than normal; artificial extension of darkness beyond sunrise will delay ovulation.
7. The gonads, particularly the testes, are organs of some complexity. In them the gametes ripen in fairly rigid succession of generations. There is an unexplained, apparently inherent rhythm underlying the twice daily sperm and egg release, even though the exact timing of these events is influenced by the rising and setting of the sun.

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